
NOTES ON THE VASCULAR ANATOMY OF THE FRUIT OF *TAKHTAJANIA* (WINTERACEAE) AND ITS INTERPRETATION¹

Thierry Deroin²

ABSTRACT

During fruit set the vascular skeleton of the *Takhtajania* gynoecium did not alter fundamentally, but there is a transfer of role in ovule nutrition from the median carpel bundles to meridian-lateral and partly lateral ones, in accordance with a pattern previously recognized in Annonaceae and Magnoliaceae. Lack of sclerification (only some phloem fibers) of secondary tissues and weak lengthening of stipe demonstrate the paedomorphic character of this genus among the Winteraceae. This trait is correlated with wider morphogenetic potentialities: syncarpy and likely dehiscence.

Key words: ovary, paedomorphy, paracarpy, *Takhtajania*, winteroids.

About 20 years ago, the description of bicarpellate syncarpy in *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy from Madagascar (Leroy, 1977, 1978, 1980; Vink, 1978) catalyzed a phase of research and analysis regarding the evolution of the gynoecium of Winteraceae and of Magnoliales in general. More recently, Leroy (1993) presented a brief synthesis of the debate resulting from this discovery, which was all the more interesting since the information available at the time gave him every reason to believe that the genus *Takhtajania* was extinct. However, after considerable effort, Malagasy foresters and their colleagues from the Missouri Botanical Garden found living plants of *T. perrieri* in 1997, and thereby made it possible to undertake a study of the vascular anatomy of the fruits of this species. The results presented here thus represent an unexpected continuation of an earlier study of the gynoecium of this plant (Deroin & Leroy, 1993).

MATERIALS AND METHODS

The fruits examined in this study were collected by C. Birkinshaw (483, MO) in the Anjanaharibe-Sud reserve. They were fixed in the field in FAA and then transferred in the lab to a glycerol:ethanol:water solution for storage. One fruit was dehydrated using tertiary butanol and embedded in paraffin (melting point = 60°C) using standard pro-

cedures (Gerlach, 1984), and then cut into 12 µm transverse sections, which were stained successively with 0.5% aqueous Astrablue and 10% Ziehl's fuchsin, and then dehydrated using acetone and mounted in Eukitt.

A similar protocol was used for an older flower from the type material (*Perrier de la Bâthie 10158*, P) after restoration using 10% aqueous ammonia, postfixation in FAA, and the preparation of 8 µm sections (Deroin & Leroy, 1993). Comparison of the material from these two sources was thus facilitated. Photographs were prepared using a Zeiss photomicroscope with an orange filter and Ilford Pan F Plus 50 ASA film.

OBSERVATIONS

The material available contained only the later stages of fruit development (Fig. 1). The series of fruits showed that: (1) no growth, either in length or thickness, occurs outside the gynoecium; (2) a basal stipe(s) observed in the fruit must have formed during an earlier stage, as little if any further elongation can be seen in the late series studied here, and the stipe is not yet differentiated at anthesis (Vink, 1978), although its vascularization can already be detected (Deroin & Leroy, 1993); (3) the ovary locule (l) does, however, grow considerably, and is asymmetrical in the plane of the two carpels, one of which elongates more than the other.

¹ I express my gratitude to Pete Lowry (MO) for his English translation of the original draft, Dawn Frame (Montpellier, France) for her kind help in literature and discussions, as well as the anonymous reviewer for his careful corrections and constructive remarks.

² Laboratoire de Phanérogamie, Muséum National d'Histoire Naturelle, 16, rue Buffon, F-75005 Paris, France. deroin@mnhn.fr

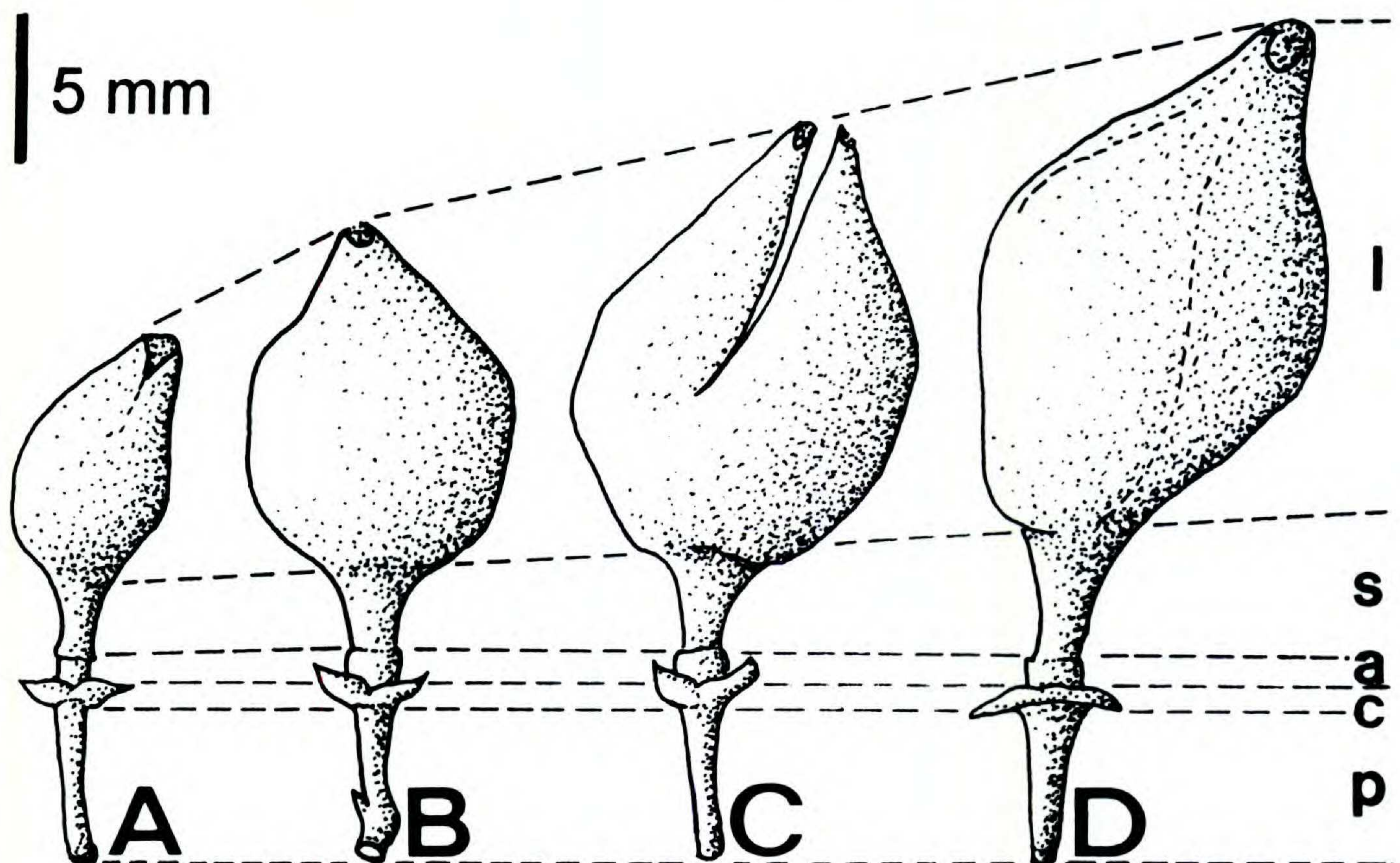


Figure 1. Four developmental stages of *Takhtajania* fruit. In the sample studied, dehiscence (C) can take place before maximum size is reached. a: androecial zone; c: calyx and corolla zone; l: area of the ovary locules; p: pedicel; s: stipe.

This differential growth could explain the rather irregular dehiscence that proceeds downward from the stigmatic scars.

The anatomy of the pedicel and receptacle of the fruit does not differ substantially from that of the flower (Fig. 2). The highly elliptic stele, with 15 vascular strands (Fig. 2A), gives rise to 2 median sepals (Fig. 2B, C), and then their lateral bundles (Fig. 2D–G). The stele, which then becomes circular and broadly medullate in cross section (Fig. 2H), gives rise farther up (Fig. 2I, J) to all of the separate traces feeding the petals and stamens (ca. 27 in all). The histological changes that take place following anthesis are minimal: lignification of the xylem (vessels stainable with fuchsin) and formation of subero-phellodermal strata at the level of the corolla and the androecium. No secondary phloem-xylem structures form.

At the extreme base of the stipe (Fig. 2K) the gynoecium appears to lack cork, and the stele divides (Fig. 2L–O) in connection with the dorsal sutures of the carpels. Higher in the stipe (Fig. 2P–S) the stele condenses in the plane of the sutures, forming two symmetrical lines of bundles. Two divided median carpellary bundles can thus be recognized, as often seen in Winteraceae, which are fused to the meridian-lateral bundles to form median complexes (mc) and the two synlateral bundles

(sl), which are relatively narrower at this level. The stele then becomes more complex (Fig. 2T) by giving rise to meridian-lateral traces, whereas the sutures extend far to the interior by a well-differentiated epidermis (Fig. 4C). The center is obliterated by large-celled parenchyma, although at anthesis this level is hollow (Deroin & Leroy, 1993, figs. 1, 3).

At the base of the locule in the fruit (Fig. 2U) the lateral and median-lateral vascularizations are much more developed than the median carpellary complexes, whereas the inverse is true at anthesis. Up to the mid-level of the locule, these divided median complexes give rise on each side to 3 median-lateral bundles (1, 2, and 3, Fig. 3A–C), whereas the lateral carpellary bundles are generally separate (Fig. 3A–E, 11, and 12).

Fruit dehiscence proceeds from the top down as the wall is torn either between the separated laterals or next to the synlateral. No specialized tissues can be seen at this level (Fig. 4F). The vascularization at the top of the fruit is formally identical to that observed in the ovary (Fig. 5A, C, E): meridian-lateral traces 1 and 2 fuse back with the laterals to form the placental bundles (pl, Fig. 5B, D). It should be noted, however, that pl2 is comprised only of the median-lateral traces 1 and 2 of carpel 2. The median-lateral traces 3 terminate

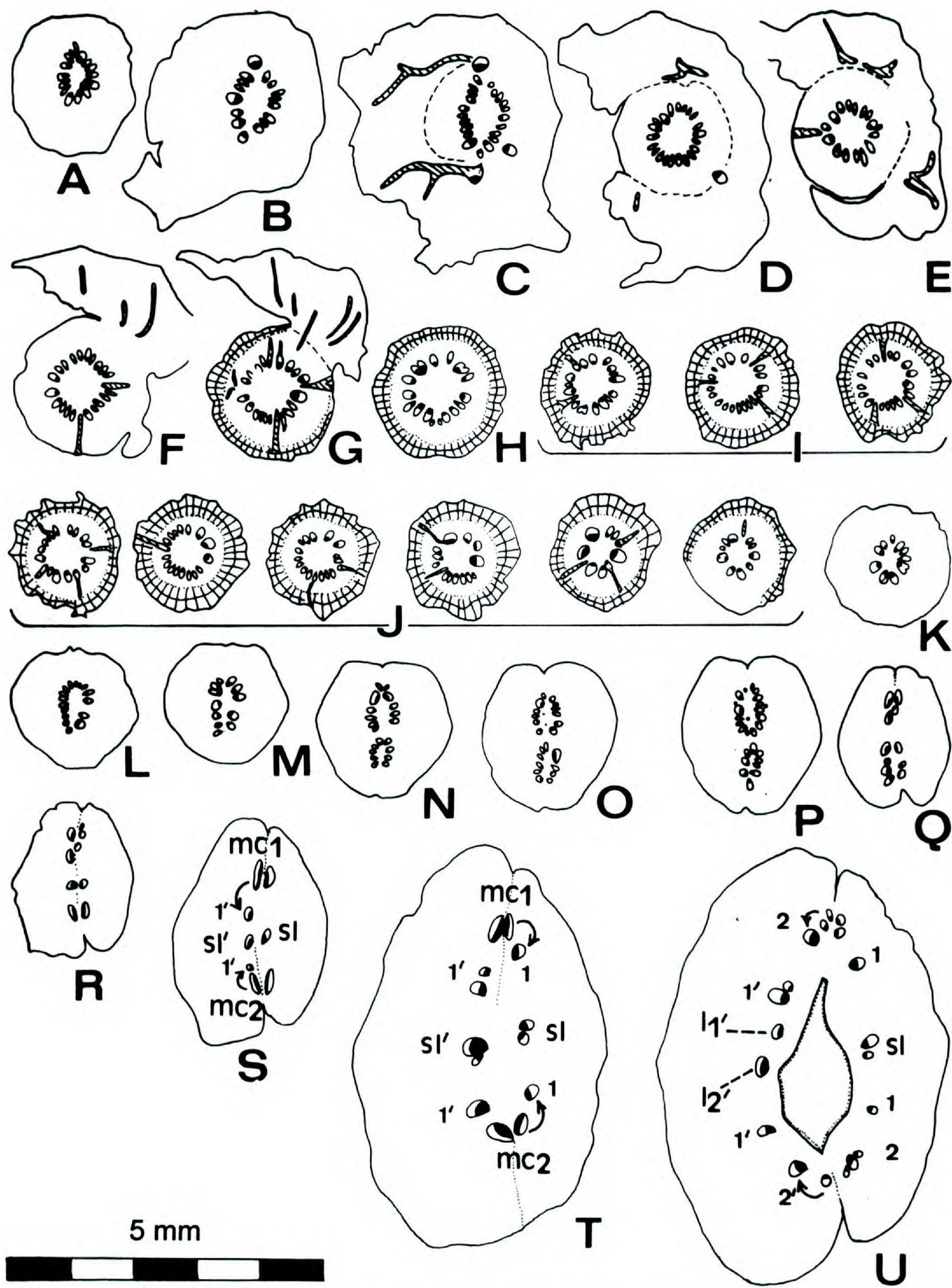


Figure 2. Ascending sections of *Takhtajania* fruit. —A. Pedicel. —B–G. Perianth. —H–J. Androecium (note the peripheral cork). —K–T. Stipe. —U. Extreme base of the ovary locule. l: lateral bundle; mc: median complex; sl: synlateral; 1, 1', 2, 2', median-laterals.

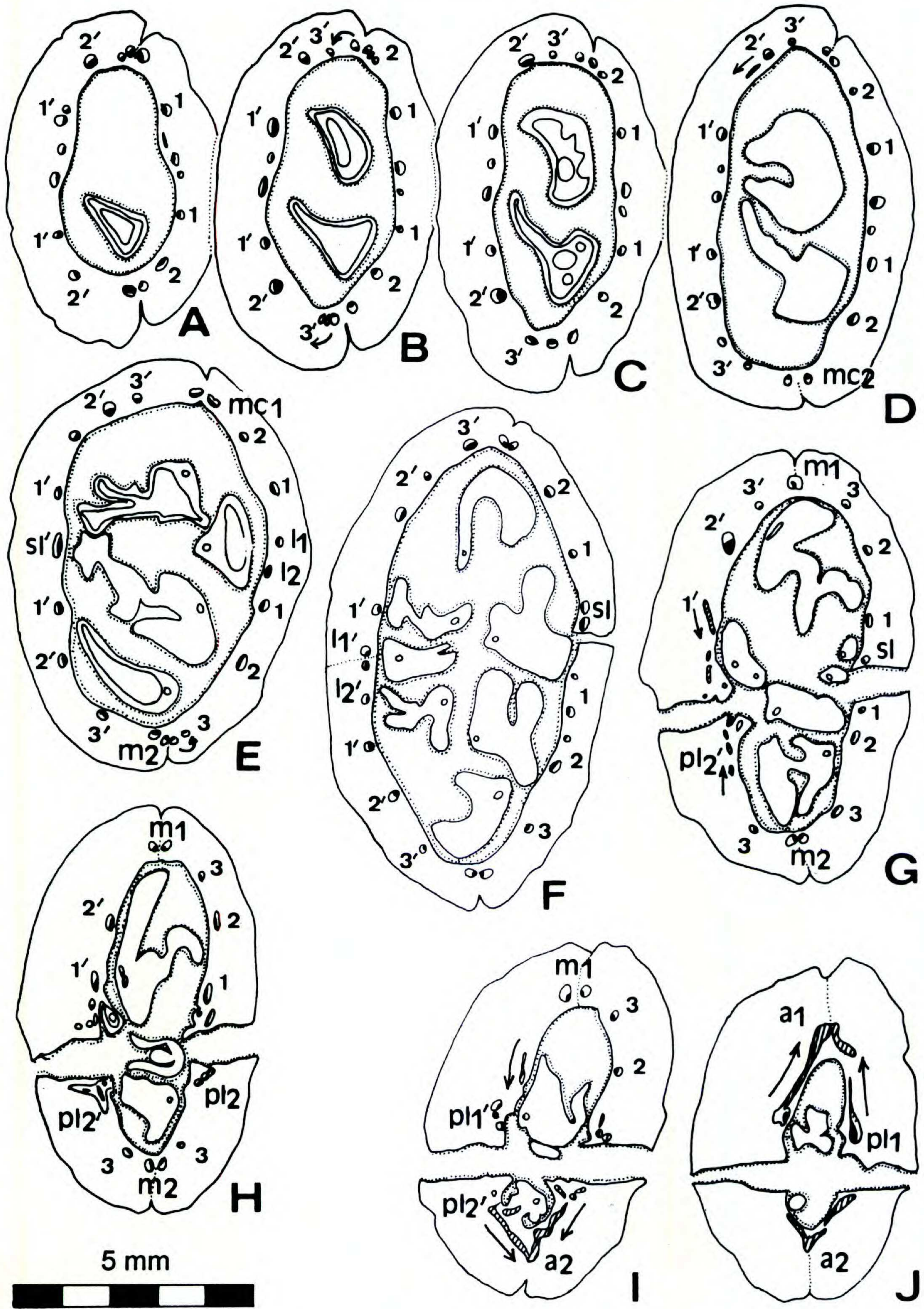


Figure 3. Ascending sections of *Takhtajania* fruit. —A–F. Lower half of the ovary. —G–J. Upper half of the ovary. a1, a2: apical vascular arcs; m: carpellary median; pl: placental bundle; 1, 1', 2, 2', 3, 3', meridian-laterals.

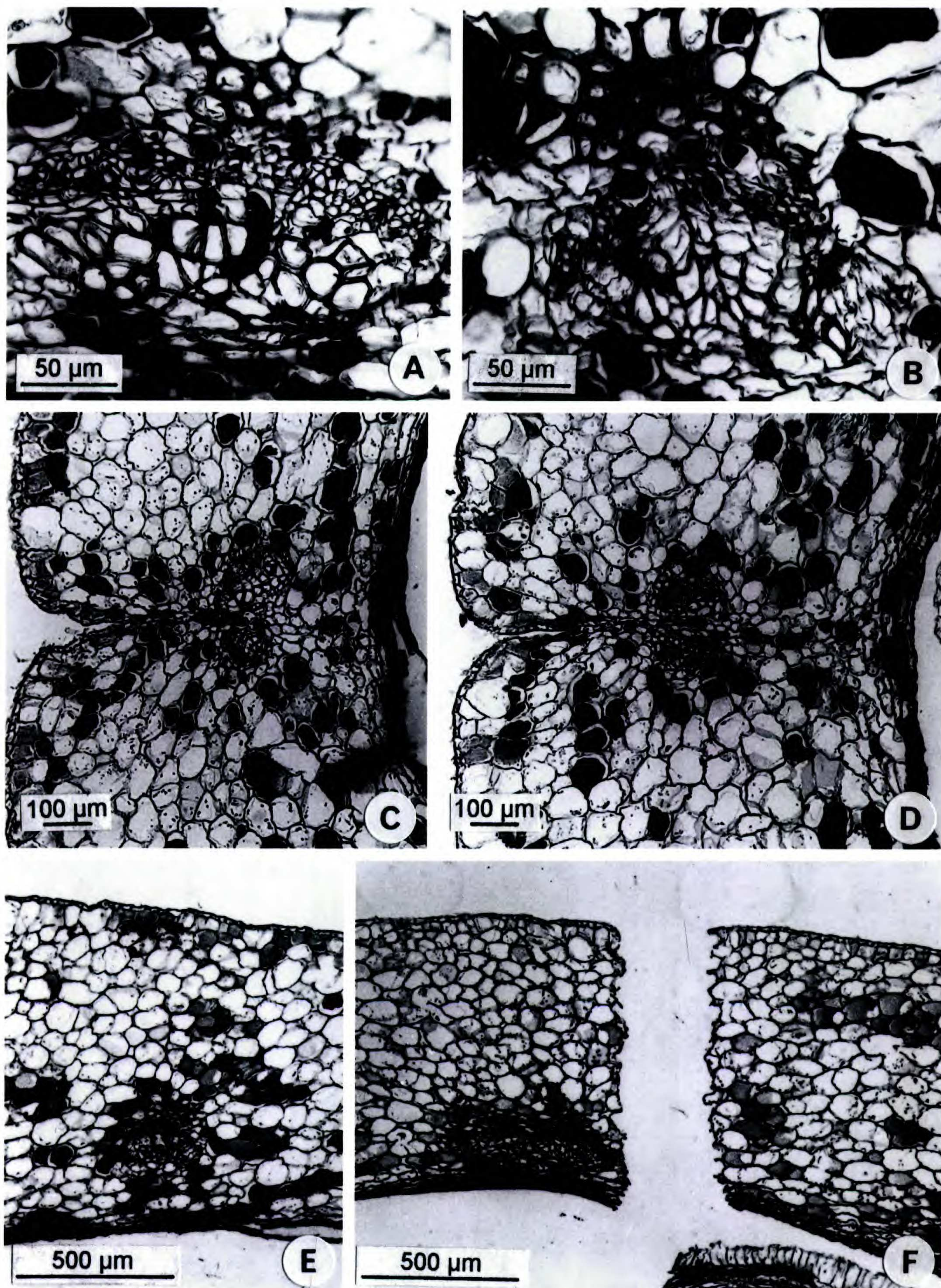


Figure 4. Histology of the fruit wall of *Takhtajania*. —A. Synlateral bundle (sl') (cf. Fig. 3E). —B. Meridian-lateral bundle 2' (cf. Fig. 3E above). —C. Transverse section of the wall at the level of the median bundle m1. —D. Transverse section of the wall at the level of the insertion of the seeds. —E. Transverse section of the wall at the level of the meridian-lateral (middle of the ovary locule). —F. Transverse section of the wall at the level of the synlateral (sl) (cf. Fig. 3F); note the unspecialized dehiscence cleft, with no epidermal boundaries.

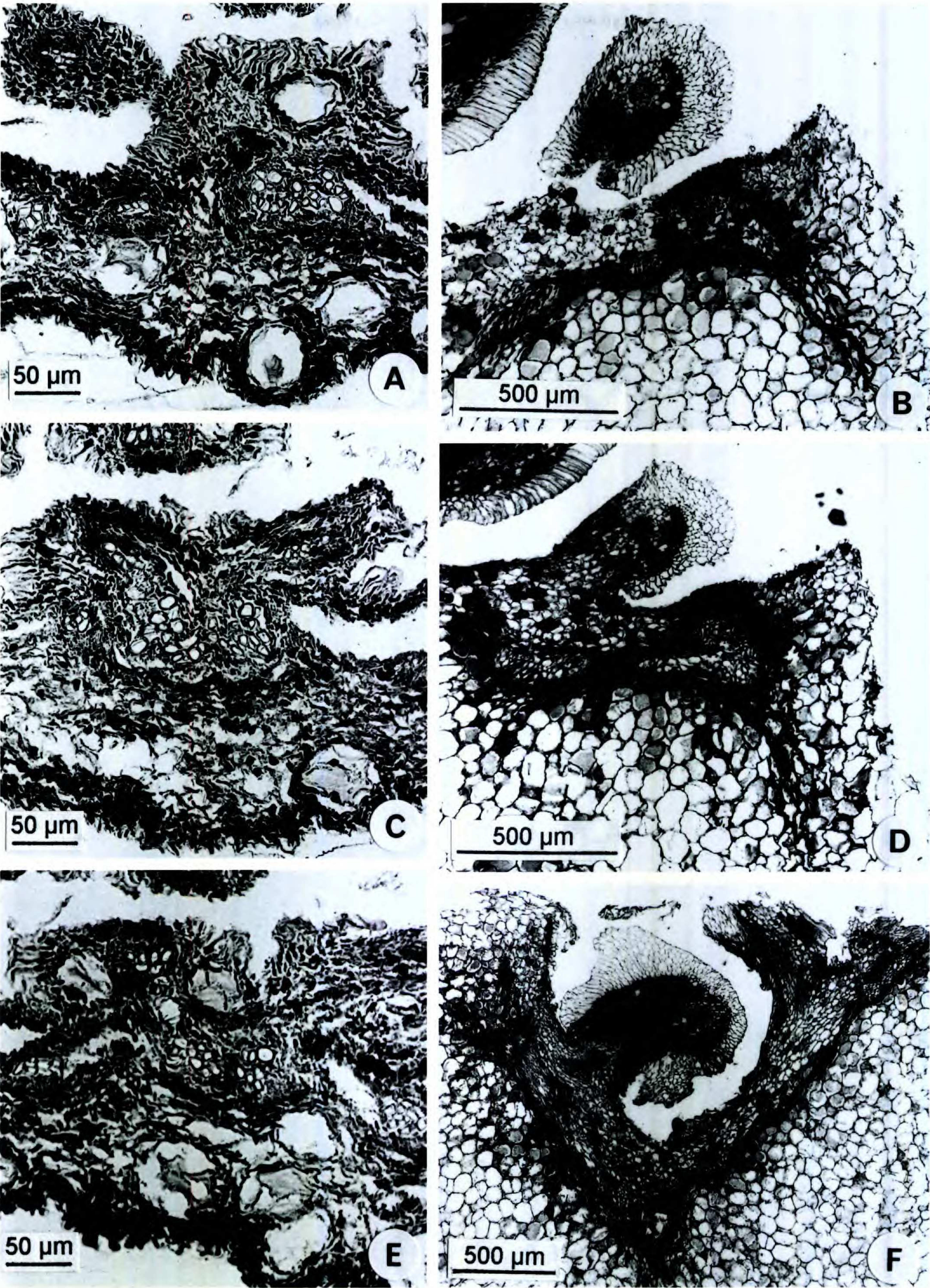


Figure 5. Placental vascularization of *Takhtajania*. —A, C, E. Ascending sections of one of the two placentas at anthesis. —B, D. Ascending sections of half a placenta at fruit stage (cf. 1', pl 1', Fig. 3H, I), dehiscence cleft visible on right. —F. Apical vascular arc a2 (cf. Fig. 3, I).

in the wall, and the placental bundles fuse back with the median bundles to form vascular arcs a1 and a2 (Figs. 3I, J, 5F). The vascularization connected to the stigmas naturally disappears along with the latter.

The histological changes of the gynoecium that take place from anthesis to formation of fruit appear to be minor. The ovary wall (Fig. 4E) shows a rather ordinary structure at mid-height: a thin epidermis that is highly fuchsin-stainable throughout the locule and covered with a fairly thick hypodermis that is almost palisadal in places; and lacunar parenchyma ca. 15 cells thick, including some fuchsin-stainable secretory cells as well as cells with calcium oxalate crystals, but lacking sclerified cells. The vascular bundles are surrounded by a crown of highly fuchsin-stainable cells and are largely comprised of metaxylem and metaphloem that are separated by 3 to 5 layers of cambial cells. The protoxylem is crushed and the protophloem is transformed into sclerified fibers with a lumen that is sometimes narrow (Fig. 4A, B). No secondary formations are present in the gynoecium.

At all levels the external epidermis clearly extends to the center of the divided medians (Fig. 4C, D).

DISCUSSION

On the basis of this study, five important features of the fruit of *Takhtajania* can be identified:

(1) Winteraceae appear to be characterized in general by floral vascularization that lacks a cortical system, and in which secondary vascular structures are absent (Nast, 1944; Deroin & Frame, 1998). By contrast, lignification of the xylem and the appearance of phloem fibers are clearly delayed in *Takhtajania*, as compared, for example, to *Exospermum* or *Zygogynum* (Deroin & Frame, 1998), in which differentiation occurs at anthesis. The total absence of sclerenchyma in *Takhtajania*, especially in the fruit wall, appears to be unique within the family, based on the work of Harvey (1982: 164). This type of unspecialized histology is also found in the syncarpous gynoecia of Annonaceae–Monodoroideae (Deroin, 1997), although at anthesis.

(2) The evolution of a stipe, as in most species of *Bubbia* (Winteraceae) (Harvey, 1982: 169), makes it easier to interpret the vascularization at the base of the gynoecium and thus its morphology. In the lower half of the gynoecium the stele divides to form two closed steles (Fig. 2K–P), which in fact clearly represent an incomplete fusion of the peltate bases of the two carpels. This peltation was not

visible at anthesis (Deroin & Leroy, 1993) and only appears later in the development of *Takhtajania*, whereas it is rather frequent and probably primitive in other Winteraceae (Leinfellner, 1965, 1966a, 1966b; Endress, 1994; Igersheim & Endress, 1997).

Several very small, isolated bundles are found in the medullary parenchyma (Fig. 2O–P) of the fruit that are not yet present at anthesis, but which are likely homologous with the basal plexus of the gynoecia in Annonaceae and even in Magnoliaceae (Deroin, 1999). Higher up, the steles open once again toward one another while folding back around the median complexes, which divide, as is generally the case in Winteraceae.

(3) From a strictly vascular perspective, the syncarpy found in *Takhtajania* is not particularly advanced, as confirmed by the organization of the stipe and the synlateral bundles, which are often disjointed (Fig. 2U, I1', and I2'; Fig. 3E, I1, and I2). In this respect the situation is similar to that found in *Monodora brevipes* Benth. (Annonaceae) (Deroin, 1997).

(4) Fruit dehiscence in the material studied is initially difficult to interpret, as the splitting could have resulted from the dehydrating action of the fixative, which would account for the irregularity of the opening. Nevertheless, the simple fact that this phenomenon can occur is of interest, as no other syncarpous member of Magnoliales has dehiscent fruits. Moreover, the possible occurrence of dehiscence in the mericarps of apocarpous Winteraceae is still poorly understood (Harvey, 1982: 167), and was not reported for members of the family in New Caledonia (Vink, 1993).

On the contrary, differentiation of the epidermis between the halves of the divided median bundles suggests primitive dehiscence at this level, which is in fact still functional in many Magnoliaceae and even some archaic Annonaceae such as *Anaxagorea* and *Xylopia*. The presence of apical vascular arcs (Fig. 3I, J, a1, and a2) linking the placental zones, which are present in all Winteraceae studied to date (Bailey & Nast, 1943; Nast, 1944; Ueda, 1978; Harvey, 1982) except *Drimys* (Tucker & Gifford, 1964; Tucker, 1975; Doweld, 1996), does not, however, favor dorsal dehiscence.

(5) The vascular rearrangements during fruit development (Fig. 6) confirm that the nutritive role is transferred from the median carpellary bundle (A) to the lateral bundles (B), a phenomenon that appears to be very generalized among Annonaceae (Deroin, 1997) and Magnoliaceae (Deroin, 1999). The strengthening in the fruit of the meridian-lateral bundles of the first and second orders (Fig. 6,

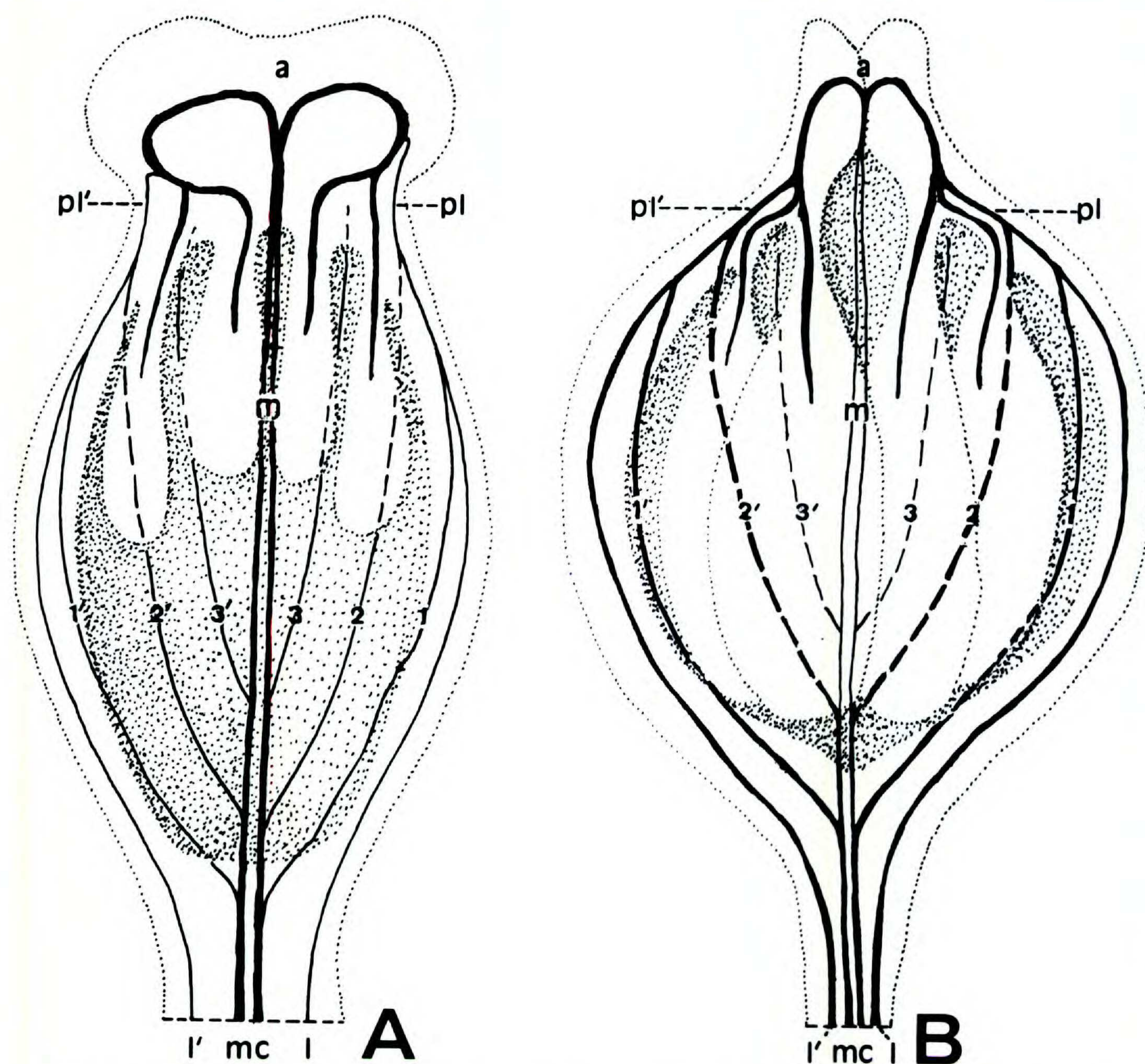


Figure 6. Interpretive schemes of transfer of vascular functionality in the gynoecium of *Takhtajania* from anthesis (A) to mature fruit (B). a: apical vascular arc; l, lateral; m, median; mc, median complex; pl, placental bundle; 1, 2 and 3: median-lateral bundles.

1, 1', 2, 2'), which then contribute in large part to the apical placental bundles (Fig. 6, pl', pl), leads to a vascular pattern that is functionally comparable to that of the carpel in *Ambavia gerrardii* (Baill.) Le Thomas, a rather primitive Malagasy Annonaceae, in which the ovules are fed by the meridian-lateral traces (Deroin & Le Thomas, 1989).

In the case of *Takhtajania* (Fig. 6B), eight functional meridian-lateral bundles (four per carpel) feed the eight seeds, with an additional role being played by the synlateral traces. The remaining four meridian-lateral traces, of the third order, most likely represent the relictual vascularizations of four additional ovules. While the closely related genus *Bubbia* has an average of 12 ovules per carpel (Harvey, 1982: 172), the genus *Takhtajania* appears to have undergone a reduction in the number of

ovules (to 4 per carpel in the material studied), perhaps following fusion of the carpels. This reduction does not appear to have occurred in the syncarps of Annonaceae–Monodoroideae (Deroin, 1991), and instead results from the distinctive placentation of Winteraceae.

CONCLUSIONS

Study of the fruit of *Takhtajania* shows that only modest changes have taken place in the organization of the ovary, but that they are nevertheless very instructive. The latent peltation of the carpels and the formation of a very short stipe resemble the tricarpellate apocarpous gynoecium of *Anaxagorea luzonensis* A. Gray, a primitive Annonaceae (Deroin, 1988), and thus suggest the early appearance

of syncarpy as in Annonaceae–Monodoroideae (Derooin, 1991), which preserves in some way the primitively open carpel structure. The vascularization of the seeds shows a delayed pattern which parallels that of the ovules of the syncarpous gynoecium of Canellaceae (Leinfellner, 1967); the gynoecium of *Takhtajania* is thus paedomorphic, with a carpel pattern very near that of *Bubbia*, a rather few-carpelled and thus archaic genus (1 to 11 carpels after Harvey, 1982: 148). In these two genera the dehiscence was at first dorsal, as demonstrated by the epidermal differentiation at the median trace level, the ventral dehiscence being restored in *Takhtajania* because of the imperfection in vascular syncarpy. By comparison, in Annonaceae–Monodoroideae, all with indehiscent fruits, however, vascular syncarpy is yet more advanced in *Isolona* than in *Monodora*, sometimes even with an ultimate reduction and disappearance of synlateral traces (Derooin, 1997). When considering the continuum from the flower to the fruit, this morphological and functional convergence among Gondwanan Magnoliales appears to highlight their shared dispersal syndrome, likely due to common paleoenvironmental constraints.

As a final remark, it is noticeable that the main vascular rearrangements during the fruit set are located in the winteraceous gynoecium only, while the regular receptacle vasculature too is subject to disruptions in Magnoliaceae and Annonaceae, in close relation with seed nutrition (Derooin, 1999).

Literature Cited

- Bailey, I. W. & C. G. Nast. 1943. The comparative morphology of the Winteraceae. II—Carpels. *J. Arnold Arbor.* 24: 472–481.
- Derooin, T. 1988. Aspects anatomiques et biologiques de la fleur des Annonacées. Unpublished Thesis, University of Paris 11, Orsay, 590.
- . 1991. Distribution of stigmatic plate patterns and evolution in Annonaceae. *Compt. Rend. Acad. Sci. Paris, Sér. 3, Sci. Vie* 312: 561–566.
- . 1997. Confirmation and origin of the paracarpy in Annonaceae, with comments on some methodological aspects. *Candollea* 52: 45–58.
- . 1999. Functional impact of the vascular architecture of flowers in Annonaceae and Magnoliaceae, and its bearing on the interpretation of the magnoliaceous gynoecium. *Syst. Geogr. Pl.* 68: 213–224.
- & D. Frame. 1998. Flower vasculature in Magnoliaceae, Annonaceae and Winteraceae: Lessons in evolutionary constraints. The First International Symposium on the Family Magnoliaceae, Guanzhou, P. R. China, Abstracts: 26.
- & J.-F. Leroy. 1993. On the interpretation of ovary vascularization in *Takhtajania* (Winteraceae). Some anatomical characters related to the magnoliatean paracarpy. *Compt. Rend. Acad. Sci. Paris, Sér. 3, Sci. Vie* 316: 725–729.
- & A. Le Thomas. 1989. On systematics and evolutive potentialities of Annonaceae: Case of *Ambavia gerrardii* (Baill.) Le Thomas, an endemic Malagasy species. *Compt. Rend. Acad. Sci. Paris, Sér. 3, Sci. Vie* 309: 647–652.
- Doweld, A. B. 1996. On the origin of the carpel as evidenced by its vascular skeleton. *Phytomorphology* 46: 387–394.
- Endress, P. K. 1994. *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge Univ. Press, Cambridge.
- Gerlach, D. 1984. *Botanische Mikrotechnik*, ed. 3, Thieme, Stuttgart.
- Harvey, W. J. 1982. Systematic Studies in *Bubbia* Van Tieghem and Related Genera. Unpublished Thesis, University of Reading.
- Igersheim, A. & P. K. Endress. 1997. Gynoecium diversity and systematics of the Magnoliales and winteroids. *Bot. J. Linn. Soc.* 124: 213–271.
- Leinfellner, W. 1965. Wie sind die Winteraceen-Karpelle tatsächlich gebaut? I—Die Karpelle von *Drimys*, Sektion *Tasmannia*. *Österr. Bot. Z.* 112: 554–575.
- . 1966a. Wie sind die Winteraceen-Karpelle tatsächlich gebaut? II—Über das Vorkommen einer ringförmigen Plazenta in den Karpellen von *Drimys*, Sektion *Wintera*. *Österr. Bot. Z.* 113: 84–95.
- . 1966b. Wie sind die Winteraceen-Karpelle tatsächlich gebaut? III—Die Karpelle von *Bubbia*, *Belliolum*, *Pseudowintera*, *Exospermum* und *Zygogynum*. *Österr. Bot. Z.* 113: 245–264.
- . 1967. Über die Karpelle verschiedener Magnoliales. V. *Pleodendron* (Canellaceae). *Österr. Bot. Z.* 114: 502–507.
- Leroy, J.-F. 1977. A compound ovary with open carpels in Winteraceae (Magnoliales): Evolutionary implications. *Science* 196: 977–978.
- . 1978. Une sous-famille monotypique de Winteraceae endémique à Madagascar: Les Takhtajanioideae. *Adansonia*, ser. 2, 17: 383–395.
- . 1980. Nouvelles remarques sur le genre *Takhtajania* (Winteraceae–Takhtajanioideae). *Adansonia*, ser. 2, 20: 9–20.
- . 1993. Histoire des travaux sur le *Takhtajania*. Pp. 109–119 in *Origine et Évolution des Plantes à Fleurs*. Masson, Paris.
- Nast, C. G. 1944. The comparative morphology of the Winteraceae. VI—Vascular anatomy of the flowering shoot. *J. Arnold Arbor.* 25: 454–466.
- Tucker, S. C. 1975. Carpellary vasculature and the ovular supply in *Drimys*. *Amer. J. Bot.* 62: 191–197.
- & E. M. Gifford. 1964. Carpel vascularization of *Drimys lanceolata*. *Phytomorphology* 14: 197–203.
- Ueda, K. 1978. Vasculature in the carpels of *Belliolum pancheri* (Winteraceae). *Acta Phytotax. Geobot.* 29: 119–125.
- Vink, W. 1978. The Winteraceae of the Old World. III. Notes on the ovary of *Takhtajania*. *Blumea* 24: 521–525.
- . 1993. Winteraceae. In: P. Morat & H. S. Mackee (editors), *Flore de la Nouvelle-Calédonie* 19: 90–171. Muséum National d'Histoire Naturelle, Paris.